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PATENT APPLICATION

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:

Applicant: Trogolo et al.

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Title: Encapsulated Inorganic Antimicrobial
Additive for Controlled Release

Examiner: F. I. Choi

Art Group: 1616

DECLARATION OF JEFFREY A. TROGOLO

PURSUANT TO 37 CFR 1.132

I, Jeffrey A. Trogolo, hereby declare as follows:

I am Chief Technology Officer for AgION Technologies, Inc. and have held this position, in AgION Technologies, Inc. and its predecessor entities, since February of 1998.

I hold a Ph.D. in Materials Science and a B.S. in Materials Engineering from Rensselaer Polytechnic Institute in Troy, NY. I have published more than 10 papers in various journals including the Journal of Materials Science, the Proceedings of the Society for Biomaterials and the Proceedings of the Materials Research Society.

I have more than ten years experience in the field of antimicrobial modification of polymers and continue to have a hands-on research role investigating the capabilities and mode of activity of antimicrobial agents, especially the AgION silver zeolite antimicrobial agent, in various polymer compositions and coatings.

I am a co-inventor of the invention disclosed in and embraced by the claims of the above-referenced pending US patent application. I am aware of the arguments being put forth in rejecting this application and, in particular, those relating to Japanese Patent Publication No. 4-66512 and its supposed teachings.

In response to the assumptions and conclusions reached by the Patent Office in the prosecution of this application, particularly in relation to JP 4-66512, I wish submit the following experiments in rebuttal to the assumptions and as evidence of unexpected results and to make the following statements and observations based on my extensive experience in the art. All experiments presented herein were conducted by me or under my direct supervision.

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Particle Size Effect

This first experiment was conducted to demonstrate the marked and unexpected benefit of increased particle size on ion-release. Here, a hydrophilic acrylic polymer encapsulated silver zeolite antimicrobial was prepared by adding 50 parts by weight of a silver zeolite antimicrobial agent (AgION AJ10D antimicrobial) to 50 parts by weight (based on the solids) of an encapsulating curable hydrophilic acrylic prepolymer mix (AEP-50 from IH Polymeric Products of Sandway, Kent, UK). The mixture was allowed to cure and the resultant polymer was found to have the zeolite particles fairly evenly distributed throughout the polymer. The polymer material was then ground and the resultant particles classified according to the following particle size ranges <53 μ , 53 μ to 106 μ , 106 μ to 212 μ , and > 212 μ . Five polymer formulations were prepared by dry blending 1% by weight AgION AJ10D antimicrobial zeolite, and 2% by weight of each cut of the hydrophilic acrylic polymer encapsulated antimicrobial agent in separate samples of ultra high molecular weight polyethylene (UHMWPE - a hydrophobic polymer) powder to attain a final concentration of 1% AgION antimicrobial zeolite in each polymer formulation. Dry blending was performed for a sufficient period of time to ensure a substantially homogenous dispersion of the antimicrobial additive in the UHMWPE powder. Aliquots of each polymer formulation were then compression molded into a 2mm thick sheet from which test coupons measuring approximately 5 cm x 5 cm x 2 mm thick were cut.

Four samples of each test coupon were then serially extracted in an 0.8M NaNO₃ test solution to determine the quantity and kinetics of silver release. Extraction was initiated by submerging each test coupon in 100 ml of the test solution. After a soak time of one or more days, the test coupon was removed and the solution tested for silver concentration using a Graphite Furnace Atomic Absorption Spectrometer (PerkinElmer AAnalyst 600). The same test coupon was then placed in a fresh 0.8M NaNO₃ solution and the process repeated. Those test coupons containing the AgION antimicrobial zeolite in neat form were subjected to 11 extractions over 22 days; whereas, those test coupons containing the microencapsulated antimicrobial were subjected to 25 extractions over 111 days. The quantity (μ g) of silver depleted from each sample on each extraction was calculated as the product of [Ag⁺] (μ g/L) and the test volume (L) divided by the surface area of the test coupon so as to provide a normalized silver release per square centimeter of surface area. Tables 1 and 2 present the average extraction results for the four samples, the numbers presented representing the cumulative mass density of silver released. For simplicity, the results are also presented in graph form in Figure 1, which plots the cumulative Ag mass density (ng/cm²) vs. cumulative extraction time. The results for the particle size +106/212 cut are not shown in Figure 1 in order to avoid confusion inasmuch as an inconsistency was noted with respect to what was expected. Specifically, the results would suggest that samples/test results were reversed as between the +53/-106 cut and

Table 1 – Cumulative Ag Mass Density of the neat silver zeolite

Time (hours)	0	24	48	72	120	192	240	288	360	408	432	528
Ag Mass Density (ng/cm ²)	0	64	81	87	93	98	100	102	104	106	107	108

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Table 2 – Cumulative Ag Mass Density (ng/cm²) of microencapsulated silver zeolite

Sample	Time (hours)														
	23	46	72	144	168	216	313	333	383	552	667	694	716	813	840
Cut Size (μ)	23														
<53	37	54	57	70	88	105	123	130	157	186	197	202	213	219	222
+53/-106	117	159	156	169	198	225	249	259	297	333	356	361	381	399	406
+106/-212	38	62	59	79	95	118	147	153	186	230	252	272	292	318	331
+212	145	307	338	414	460	515	592	615	665	764	813	853	873	903	926

Table 2(cont'd) – Cumulative Ag Mass Density (ng/cm²) of microencapsulated silver zeolite

Sample	Time (hours)													
	862	977	1052	1152	1488	1537	1702	2018	2066	2160	2185	2422	2521	2662
Cut Size (μ)	862	977	1052	1152	1488	1537	1702	2018	2066	2160	2185	2422	2521	2662
<53	224	228	238	240	247	244	250	254	254	258	258	259	260	263
+53/-106	407	418	439	448	467	466	483	496	496	508	509	513	516	523
+106/-212	330	359	394	419	469	474	531	569	565	604	611	633	639	673
+212	919	958	988	1015	1089	1088	1137	1183	1181	1208	1216	1240	1247	1273

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the +106/-212 cut through the first 20 or so extractions. Nevertheless, even if one averaged the two in order to provide results for the +53/-212 cut, it is clear that the plot would have followed a path similar to and intermediate of the plots for the +212 cut and the -53 cut.

The results shown in Tables 1 and 2 and Figure 1 demonstrate that the incorporation of a hydrophilic polymer having encapsulated therein multiple particles of an antimicrobial agent into a hydrophobic polymer matrix provides a markedly and surprisingly greater and longer-lived silver release as compared to use of an equivalent weight of the neat antimicrobial agent. Furthermore, the results set forth in Table 2 and Figure 1 demonstrate that increasing the particle size of the antimicrobial additive particles markedly improves the performance thereof even though the same amount of antimicrobial agent is present in the overall formulation. Thus, given environmental and cost concerns, the present invention allows one to use less of the environmentally sensitive and costly antimicrobial metals while achieving greater antimicrobial performance.

In following, the results above contrast sharply with the findings shown in JP 4-66512 wherein the larger particle size polyurethane coated antimicrobial agent (3 wt% coated) manifested significantly less silver ion release than those coated with just 1.5 wt% polyurethane despite the fact that both contained the same amount of silver. Furthermore, given the minimal change in particle size as between the two polyurethane coated samples, at best one would have expected an equivalent ion release: certainly not a dramatic drop as was realized.

Hydrophilic Modification: Encapsulation v. Matrix Modification.

A second experiment was conducted to demonstrate the criticality of the physical form of the invention as claimed. In this experiment, 10 grams of a silver zeolite (AgION AJ10D antimicrobial) was thoroughly mixed into 50 grams of two curable polyurethane compositions together with 3 % by wt of aziridine cross-linker. One polyurethane was a hydrophobic polyurethane and the other an 80:20 mix (by weight) of the hydrophobic polyurethane and polyvinylpyrrolidone, both from Coatings2Go of Carlisle, MA. The incorporation of the polyvinyl pyrrolidone renders the previously hydrophobic polyurethane hydrophilic. Both resin dispersions were poured into shallow trays and placed in a 103°C oven overnight so as cure/polymerize the polyurethane. The resultant solid films were individually ground in an attritor mill and screened using a 50mesh screen to remove the larger particles (i.e., those greater than 300μ). An additional quantity of virgin (i.e., no antimicrobial agent) particles of each of the two polyurethane resins was made in the same way with the exception that no antimicrobial additive was included as well.

A series of epoxy compositions were then prepared incorporating each of the aforementioned polyurethane particles. The epoxy resin system, a hydrophobic system in its cured state, was a two-part system based on diglycidyl ether of Bisphenol A and a cycloaliphatic amine curing agent. The formulation of each epoxy composition was as set forth in Table 3. All compositions, with the exception of the control, were formulated so as contain 25% by wt. silver zeolite in the cured epoxy polymer. Dispersions of each composition were prepared by hand mixing. The substantially homogeneous dispersions were then poured into individual 2-inch diameter trays and allowed to cure overnight at room temperature. The cured epoxy samples were removed from the trays and lightly sanded with emery paper and placed in individual vials containing 40 ml of an 0.8M sodium nitrate solution. The vials were placed on a tilt agitator at room temperature and rocked for 24

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hours. Thereafter, the solutions were collected and diluted 50:1 with purified water and assayed for silver using atomic absorption (AA) spectrophotometry. The results of the extraction study were as set forth in Table 3.

The results presented in Table 3 clearly demonstrate the marked and unexpected increase in silver ion release resulting from the use of an antimicrobial additive wherein the additive comprises discrete particles of a hydrophilic polymer having dispersed therein the true antimicrobial active agent. Indeed, Example 5, which embodies the invention, has nearly a 100% increase in ion release over the neat antimicrobial agent despite the fact that both contain the same amount of the same silver zeolite. Merely adding the silver zeolite together with a hydrophilic polymer as individual components to the hydrophobic matrix resin (Example 4) had essentially no effect, if not a slightly detrimental effect, on ion release despite the fact that the addition of a hydrophilic polymer to a hydrophobic polymer is a known and accepted method of increasing the hydrophilic properties of a hydrophobic polymer. The addition of hydrophobic particles having incorporated therein the silver zeolite (Example 3) and the addition of hydrophobic particles and the silver zeolite as individual components (Example 2) had no or a slightly detrimental impact on ion release as compared to the use of the neat antimicrobial agent.

Table 3

Component	Control	Example 1	Example 2	Example 3	Example 4	Example 5
Epoxy resin	25	25	25	25	25	25
AJ10D		0.5	0.5		0.5	
PU1			0.5			
PU2					0.5	
ME1				1		
ME2						1
Silver Extraction						
AA reading* (ppb)	0	8.5	6.6	7.7	7.9	15.3
[Ag] in extract [#] (ppb)	0	425	330	385	395	765

* Atomic Absorption reading of the 50:1 diluted sample.

[#] Actual silver content in the extraction solution

Water Absorption Evaluation

In a third experiment, three sets of water absorption subexperiments were conducted to demonstrate the water absorption characteristics of the neat silver zeolite, AgION AJ10D; of a hydrophilic

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acrylic encapsulated silver zeolite, comparable to that used in the particle size experiment; of a hydrophobic polyurethane; and, finally, of a hydrophilic modified version of the hydrophobic polyurethane.

Water Absorption Study #1

In the first subexperiment, a sample of the neat silver zeolite (AJ10D) was allowed to stand in an open tray in the ambient lab environment (relative humidity ~ 58%) and periodically evaluated for weight gain: weight gain being reflective of water absorption from the air. The sample evaluated was retrieved from a sealed commercial container and, thus, had no or little water content upon commencing the evaluation. The results are presented in Table 4 and demonstrate the highly hygroscopic nature of zeolites as evidenced by their ability to quickly absorb water and reach saturation/equilibrium with the ambient environment.

Table 4 – Ambient Water Absorption of Silver Zeolite AJ10D

Time (minutes)	0	30	50	93	109	151	177	206	246
% wt gain	-	6.5	9.2	12.9	13.8	15.7	16.4	17.0	17.2

Water Absorption Study #2

In the second subexperiment, a sample of a hydrophilic acrylic polymer encapsulated silver zeolite, similar to those produced in the particle size evaluation study above, was evaluated. As with the neat silver zeolite, a sample of the encapsulated silver zeolite was allowed to stand in an open tray in the ambient lab environment (relative humidity ~ 58%) and periodically evaluated for weight gain.

In this subexperiment, the microencapsulated antimicrobial was not pre-dried in order to remove ambient moisture that had already been absorbed by the materials prior to evaluation. Nevertheless, the results as presented in Table 5 demonstrate that these materials, like the neat antimicrobial agent, readily absorb water and have a tendency to reach saturation/equilibrium in a reasonably short period of time under ambient conditions.

Table 5 – Ambient Water Absorption of Encapsulated Silver Zeolite AJ10D

Time (hours)	0	24	120	216	408
Wt % water	3.23	3.98	8.66	8.48	9.07

Water Absorption Study #3

In the third and final subexperiment, the two polyurethane compositions from the Hydrophilic Modification Study set forth above were evaluated for water absorption under ambient and high humidity conditions. Test samples were prepared by placing 10 grams of each curable polyurethane

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composition in 3 inch diameter hexagonal boats and allowing the same to cure/dry overnight in a 75°C oven. The cured/dried samples were weighed and one set of each of the two polymer samples were allowed to sit out on a lab bench (relative humidity ~ 40%) and the second set placed in a closed vessel with paper towels that had been wetted (saturated) with water. Each sample was weighed periodically to assess weight gain due to water absorption. The normalized results of these evaluations are presented in Table 6.

As seen from the results in Table 6, relative humidity or ambient humidity has a notable effect on total water absorption and the rate of water absorption. Nevertheless, the results also demonstrate that all polymers, to the extent they have any degree of hydrophilic nature, including those hydrophobic polymers having weak hydrophilic properties, have an inherent tendency to absorb water in an effort to reach equilibrium (water equilibrium) with their environment. Specifically, as expected, the hydrophilic polyurethane quickly and noticeably absorbed water, even within the first hour exposure to low ambient humidity. Conversely, as also expected, the hydrophobic polyurethane absorbed only a nominal amount of water, in line with its low hydrophilic properties. Even in the high humidity environment, it was only able to absorb about 2.5 wt% water after 90 hours.

Table 6 – Water Absorption of Hydrophilic v. Hydrophobic PU

Sample	Condition	Time (hours)			
		0	1	2.5	90
Hydrophobic	Ambient	10.0	10.041	10.052	10.059
Hydrophilic	Ambient	10.0	10.085	10.142	10.249
Hydrophobic	High humidity	10.0	10.128	10.186	10.246
Hydrophilic	High humidity	10.0	10.614	10.932	11.071

The results of this subexperiment demonstrate that hydrophilic polymer compositions that have not achieved equilibrium (water equilibrium) with their environment or that have been subjected to conditions that drive water from the polymer (e.g., drying or high temperature cure conditions) will immediately commence absorption of water, if available, regardless of whether the polymer is immersed in water or set out in either a high humidity environment or even a low ambient humidity environment, in an effort to reach their natural state or, at least, water equilibrium with the ambient humidity. Polymers that do not absorb water following such drying conditions when moisture is present are in all likelihood strongly hydrophobic with no or nominal water content.

Discussion of JP 4-66512

JP 4-66512 is concerned with improving the dispersability of hydrophilic inorganic antimicrobial agents, specifically silver zeolites, in hydrophobic polymer resins. It is well known that hydrophilic inorganic additives are difficult to disperse in most any matrix due to their strongly hydrophilic nature which tends to cause the particles to agglomerate and clump together. It is also well known and a standard remedial action in the art to modify the surface of such hydrophilic particles so as to

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render the surface thereof hydrophobic. This is traditionally accomplished by either chemically reacting the surface with another chemical that alters the surface chemistry to one that is hydrophobic or by coating the surface of the particles with a hydrophobic material. Perhaps the most common materials used for this purpose are silanes; however, hydrophobic polymers are also known to be useful for this purpose. On the other hand, the use of a hydrophilic material, especially one having anything but a nominal water content, for this purpose is contraindicated since a hydrophilic material would defeat and frustrate the very objective of sought by JP 4-66512, i.e., altering the surface so as to remove water and the affinity for water so as to prevent agglomeration.

The Patent Office has asserted that the polyurethane employed in JP 4-66512 is hydrophilic, but has provided no proof or support for this assertion. While an evaluation of the polyurethane of JP 4-66512 or even a review of its literature, particularly that pertaining to the Nippolane 1100, the component most likely responsible for any hydrophilic characteristics, if any, would settle the matter, efforts on behalf of the undersigned to obtain such samples or information have not been successful. Thus, one must look to the specific teachings and results presented in JP 4-66512 and the state of the art in light of certain analogous studies, as presented above, in order to ascertain whether the polyurethane is indeed hydrophilic, and, if so, whether it is hydrophilic within the meaning of the claimed invention. It is my conclusion that it is not.

Foremost among the factors dictating that it is not hydrophilic is the state of the art, which teaches the use of hydrophobic materials as the appropriate surface modifier or treatment for hydrophilic inorganic additives for improved dispersability.

The second factor is the fact that the reference itself speaks of the need for hygroscopic materials and demonstrates the inability of its polyurethane coated particles to take up water. The process by which the coated particles of JP 4-66512 are prepared involved high temperatures, ~150°C, for an extended period of time: conditions that would tend to drive off any moisture present. Hence, in light of the results above on water absorption and the inherent characteristics of hydrophilic materials to absorb water, even ambient water, in an effort to reach its equilibrium state, one would have expected water absorption in the case of the reference's examples if they were in fact hydrophilic, even weakly so.

The third indicator is the fact that the water content was lower, 3.8% by weight, for those particles having 3% by wt. polyurethane than for those particles having just 1.5% by wt. polyurethane, 4.1% by weight water content. This result is consistent with adding a non-hydrophilic material to a hydrophilic material: in essence a dilutive effect. However, such a large drop in water content cannot be explained solely by the addition of such a small amount of material; hence, it is likely that the lower water content is also reflective of a drying of the zeolite particles during the coating process. As noted in the experimental detail of JP 4-66512, the zeolite particles were left out overnight to "sufficiently absorb humidity" prior to coating and then subjected to elevated temperatures for extended periods of time during the coating process (150°C for >20 minutes). Consequently, if not the polymer, the zeolite should have absorbed water if water were available. The fact that neither the polymer nor the zeolite absorbed water further supports the conclusion that the polyurethane was a hydrophobic polyurethane that prevented water from reaching the zeolite.

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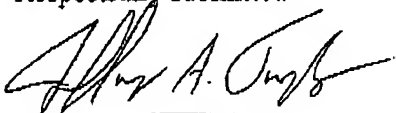
Finally, the sharp and marked drop in ion release as a result of the thicker coating (higher resin content) is also consistent with the use of a hydrophobic coating. Ion release is contingent upon water pathways for the silver ions and the ions to be exchanged therewith. In hydrophilic resins, water within the polymer provides the pathways for ion-exchange and release of silver ions. In hydrophobic resins, the pathways are provided by the natural porosity or interstitial spaces within the polymer matrix. Thus, only those antimicrobial agents that contact the outer surface of the polymer matrix or that contact a pore or interstitial space that is also in contact with the outer surface of the polymer have the potential to release ions. The ions cannot travel through the polymer matrix itself. The coating method employed in JP 4-66512 which involves the gradual unblocking of the isocyanate and consequent reaction with the polyol, effectively produces a sequential coating of the zeolite particles on essentially a molecular level. As each successive layer builds, more and more of the pores or interstitial spaces in the underlying layers are covered or blocked by the new layers. Thus, as the coating gets thicker and thicker, even on the order of tenths of a micron and less, the potential for ion release drops until the only release possible is that arising from those exposed particles at the surface. Here, the drop in ion release from 26.5 $\mu\text{g/l}$ to 17.6 $\mu\text{g/l}$ with an increase in coating thickness of no more than 0.05 μ can only be attributed to the use of a hydrophobic coating.

Conclusion

The foregoing experiments and discussion can only lead to the conclusion that the polyurethane employed in the reference, JP 4-66512, is non-hydrophilic: at least not hydrophilic as defined by and required by our invention. Furthermore, the results presented above manifestly demonstrate the unexpected and marked improvement associated with our invention. Such results are in no way anticipated by or even remotely suggested by the reference, regardless of whether the Patent Office is correct in its assertion that the polyurethane is hydrophilic.

I hereby state that all statements made herein of my knowledge are true, all statements made herein on information and belief are believed to be true and all statements made herein are made with the knowledge that whoever, in any matter within the jurisdiction of the Patent and Trademark Office, knowingly and willfully falsifies, conceals, or covers up by any trick, scheme or device a material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false wiring or document knowing the same to contain any false, fictitious or fraudulent statements or entry, shall be subject to the penalties set forth under 18 USC 1001, and that violations of this paragraph may jeopardize the validity or enforceability of any patent resulting therefrom.

Respectfully submitted



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